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ORIGINAL RESEARCH ARTICLE

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Impact of processing on the in vitro protein quality, bioactive compounds, and antioxidant potential of 10 selected pulses

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Abstract

Pulses are consumed worldwide with different processing methods, which may impact their digestibility, protein quality, and composition. This study aims to analyze the effect of extrusion, baking, and cooking on protein nutritional parameters; bioactive compounds; and the impact on antioxidant capacity (AOX) of 10 selected pulses. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed that thermal processing causes modifications to the main storage proteins in pulses. Heating decreased saponin content from 12% to 44% in most heat-processed samples; phytates were reduced 30%–84%, and polyphenol content decreased 28%–66%. In addition, the *in vitro* protein digestibility (IVPD) was enhanced 2.5%–9.5%, 3.5%–10.7%, and 2.2%–8.4% by extrusion, cooking, and baking, respectively. AOX showed an improvement in all processed samples (compared to raw flour) evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and by the oxygen radical absorbance capacity (ORAC). Fe^{2+} chelation showed that extruded and baked chickpea exhibited a decrease in IC_{50} by 40% and 70%, respectively. Extruded green and yellow split pea presented the highest Fe^{2+} chelation, improving by 11%–17% and 13–80%, respectively, when compared to the raw samples. Reducing power was enhanced by 26% in extruded chickpea, 18% and 29% in extruded and baked faba bean, respectively, and 50% in baked navy bean, when compared to the raw samples. Extrusion showed the highest β-carotene AOX improvements (IC_{50} 90%–96%). In this study, it was demonstrated that pulses AOX attributes can be enhanced by thermal processing; however, this will depend on the legume species and heating process applied. Furthermore, cooking seems to be the most effective thermal method to decrease saponins and phenolics, while extrusion reduced effectively phytic acid on bean samples, and cooking for the rest of pulses. All heating treatments affected positively IVPD, while the highest in vitro protein-digestibility corrected amino acid score (IVPDCAAS) values were observed for baked pulses. Employing adequate processing methods represents an effective strategy to improve the digestibility of their proteins, as well as increasing the antioxidant potential of the resulting ingredients.

KEYWORDS
antioxidant activity, bioactive compounds, in vitro protein digestibility, IVPDCAAS, nutritional parameters, processed pulses

1 | INTRODUCTION

FAO declared 2016 as the "Year of Pulses", since pulses have contributed to food security, helped to eradicate hunger, increased agricultural productivity, improved human health, and reduced soil degradation, thus contributing to the Sustainable Development Goals (SDGs) (FAO, 2016). Canada was the largest exporter of pulses in the world in 2018 for lentils and dry peas, with over 80% of its pulse production shipped internationally (Agriculture and Agri-Food Canada, 2020). In addition, an increasing number of consumers have started shifting to plant-based foods diets and reducing their meat consumption, which is corroborated by a decrease in pork and beef meat production and by a 9.4% increase in the vegan/vegetarian population (mostly under 35 years old), thus increasing the concern for more environmental, nutritious, and clean-label foods (Dyer et al., 2020; Health Canada, 2019).

Studies from the last decade have shown that pulse consumption provides a wide variety of health benefits, such as reducing the risk of cardiovascular diseases, helping in controlling diabetes, maintaining body weight, preventing cancer, and reducing inflammation (Duranti, 2006). Pulses are also known to contain a number of bioactive compounds, including lectins, saponins, enzyme inhibitors, phytates, polyphenols (tannins), and oxalate (Campos-Vega et al., 2010; Khandelwal et al., 2010; Pasqualone et al., 2020). These bioactive compounds are secondary metabolites synthetized by plants mainly as a defense mechanism against environmental adverse conditions (Abdel-Aal, 2016), while others are reserve compounds, such as defense proteins (Bowman–Birk inhibitors and Kunitz inhibitors), which are stored in seeds as energy pools in preparation for germination (Muzquiz et al., 2012). The effects of these compounds may be negative, positive, or both (Campos-Vega et al., 2010; Muzquiz et al., 2012). Thus, depending on the biochemical and physiological point of view, as well as their concentration, these could present beneficial and deleterious effects. Among the different effects that these compounds may have, one of the most important is their impact on protein digestibility and on the bioavailability of amino acids (Gilani et al., 2012). This is attributable to the fact that many of these compounds behave like protease inhibitors (Parca et al., 2018). Phenolic compounds such as flavonoids and condensed tannins are also known to have antioxidant properties (Campos-Vega et al., 2010; Xu et al., 2007). However, thermal processes have a direct effect on the profile of these bioactive compounds in pulses and, simultaneously, modify protein structure, quality, and potential bioactivity (El-Adawy et al., 2000; Patterson et al., 2016; Rathod & Annapure, 2016; Nosworthy et al., 2018). Pulses' levels of bioactive compounds vary considerably according to grain genotypes and environmental factors, as well as processing and storage conditions (Liu et al., 2020).

Traditional processing of pulses involves thermal treatments such as boiling, microwave cooking, roasting, extrusion, and baking, sometimes preceded by soaking or blanching (Pasqualone et al., 2020). The main purpose of thermal processing in pulses is to produce physical and chemical changes in seed structures, which enhance the bioaccessibility and bioavailability of their nutrients, as well as to improve their sensory properties (Ma et al., 2011). However, heating also causes substantial variations in bioactive compound profiles, as most of them are released to the soaking/cooking medium, degraded, precipitated, or complexed to other molecules present in pulses, thus impacting bioavailability, bioaccessibility, and bioactivity (Nosworthy et al., 2018). To understand changes in bioactives content in pulses, it is necessary to closely follow their fate during heating treatments (Abdel-Aal, 2016).

It is also well known that during gastrointestinal digestion, proteins are hydrolyzed to small peptides and amino acids so that these can be absorbed. Some of these peptides are able to scavenge free radicals inside the human body and combat oxidative stress (Carrasco-Castilla et al., 2012a, 2012b; Liu et al., 2020). However, scientific data reporting the antioxidant activity of peptides produced from pulses subjected to various heat treatments are scarce. This study aims to evaluate the effect of extrusion, baking, and cooking of 10 selected pulses on protein quality parameters, content of bioactive compounds, and their impact on antioxidant properties of hydrolysates produced during in vitro digestion.

2 | MATERIALS AND METHODS

2.1 | Supplies and sample preparation—flours

Pulse samples of red (RL, *Lens culinaris* Medik.) and green lentils (GL, *Lens culinaris* Medik.) were provided by SaskCan Pulse Trading (Regina, Saskatchewan), Thompsons Ltd. (Blenheim, Ontario) with an additional sample of green lentils provided by Diefenbaker Seed Processors (Elbow, Saskatchewan). Yellow (YSP, *Pisum sativum* L.) and green split peas (GSP, *Pisum sativum* L.) were provided by SaskCan Pulse Trading (Regina, Saskatchewan, Canada) and Thompsons Ltd. (Blenheim, Ontario, Canada) with an additional sample of yellow split peas provided by Diefenbaker Seed Processors (Elbow, Saskatchewan, Canada). Samples of beans: red kidney bean (RKB, *Phaseolus vulgaris* L.), black bean (BB, *Phaseolus vulgaris* L.), pinto bean (PB, *Phaseolus vulgaris* L.), and navy bean (NB, *Phaseolus vulgaris* L.) were provided by SaskCan Pulse Trading (Regina, SK, Canada), Thompsons Ltd. (Blenheim, ON, Canada), Diefenbaker Seed Processors (Elbow, SK, Canada), Hensall District Cooperative (Hensall, ON, Canada), Viterra (Bow Island, AB, Canada) and LegumexWalker (Winkler,MB, Canada).

Samples of faba beans (FB, *Vicia faba* L.) were provided by SaskCan Pulse Trading (Regina, SK, Canada). Samples of chickpeas (CC, *Cicer arietinum* L.) were provided by Saskcan Pulse Trading, Thompsons Ltd., and Viterra. Prior to processing, samples of similar legume species from different suppliers were combined and thoroughly mixed. Flours of pulses were obtained by milling using a Jacobson 120-B hammer mill (Minneapolis, MN) with screen hole size of 0.050 inch (0.127 cm), round. Milled pulses were subjected to three heat treatments as presented in Figure 1: for extrusion, flours (4.0–4.5 kg) were prepared using an Evolum 25 twin-screw extruder (Clextral Evolum® HT, Firminy, France) with a screw diameter of 25 mm and L/D ratio of 40 at 36 kg/h with a moisture addition of 0.8 kg/h. The screw speed was 650 rpm, with extrusion barrel temperatures of 30–50, 70–90, and 100–120°C. For cooking, pulse flours (4.0–4.5 kg) were soaked in tap water at a ratio of 1:4 (1.5 kg pulse:6 L water) for 16 h, with the water being changed prior to cooking. After, samples were boiled for 25–35 min and rinsed with water in a 4:1 ratio to stop cooking, then samples were freeze dried and milled for further use. For baking, each sample was mixed with 2–4 L of water in a mixer (Hobart mixer, model D300DT, Des Plaines, IL, USA) set up with a dough hook attachment, at a set speed #1 for 1.5 min followed by speed #2 for 2.5 min. Samples were baked in a preheated tunnel conveyor oven (Doyon® FC2-III, San Diego, CA, USA) at 193°C for 35 min. Then, baked pulses (4.0–4.5 kg of flour) were cooled to room temperature. Once the heat treatments were done, all samples were milled as described above.

2.2 | Amino acid profile

The amino acid analysis of samples was conducted in accordance with the Agilent method (Long, 2015). Briefly, 4 mg of protein of raw, extruded, cooked, and baked samples was hydrolyzed with 6 N HCl containing 0.1% phenol and Norvaline (as internal standard) for 24 h at 110 ± 2°C in glass tubes sealed under vacuum. The hydrolyzed samples were cooled to room temperature, and solutions were evaporated with nitrogen until dryness. Once dry, the amino acids were dissolved by the addition of 10 mM sodium borate buffer (pH 8.2, containing 0.1% [w/v] HCl) and then filtered with 0.22 µm polyvinylidene difluoride (PVDF) filters (low protein binding) (Sigma-Aldrich, USA). Analysis was performed using an Agilent Poroshell HPH-C18 reversed-phase column monitored with Agilent 1200 series HPLC system (Agilent Technologies Canada Inc., Mississauga, ON, Canada), utilizing an automatic post-column OPA and 9-fluorenylmethoxycarbonyl group (FMOC) derivatization and detection using absorbance at 338 nm. The separation was performed at a flow rate of 1.5 mL/min, employing a mobile phase of: (A) 10 mM Na_2HPO4, 10 mM Na_3B4O7, 5 mM NaN3, adjusted to pH 8.2 with HCl; and (B) ACN:MeOH:water (45:45:10, v/v/v). The elution program was as follows: 0 min 2% B, 20 min 59% B, and 25 min 2% B. All samples were performed in triplicate.

The content of tryptophan in the pulses samples was determined separately by alkali hydrolysis following the method of Yust et al. (2004), with slight modifications. Samples (15 mg of protein) were

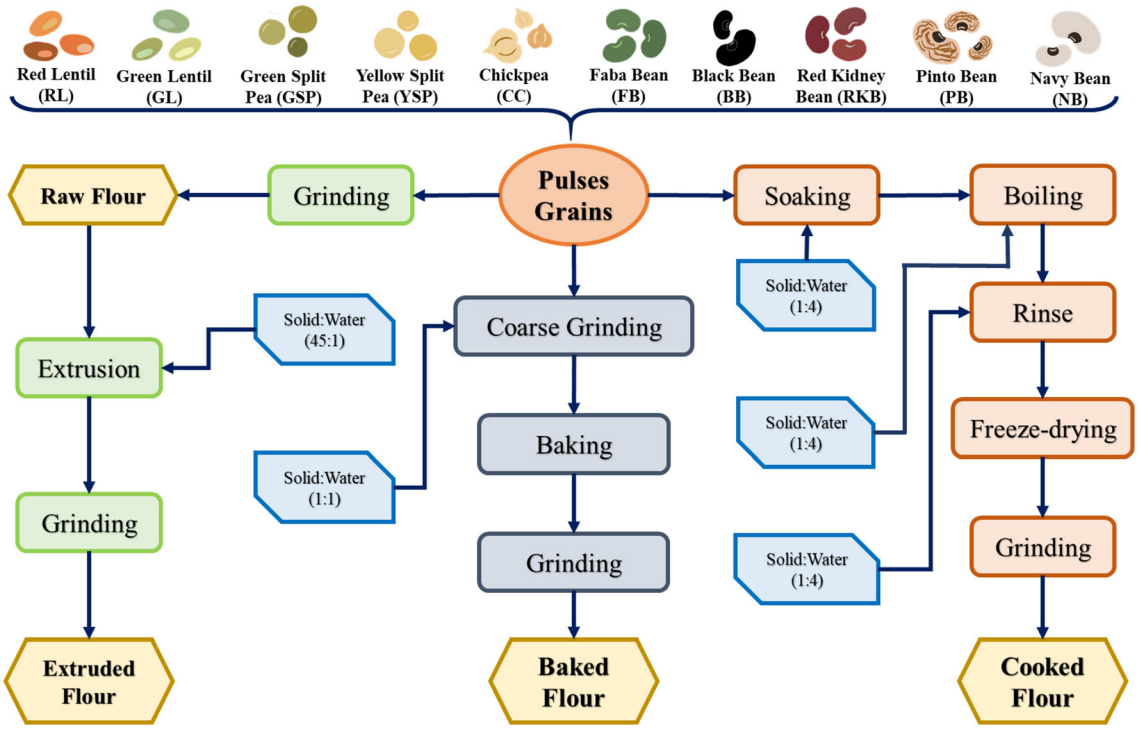

FIGURE 1 Processing methods workflow

dissolved in 3 mL of 4 N NaOH, sealed in hydrolysis tubes and incubated in an oven at 110°C for 24 h. Hydrolysates were cooled down, neutralized to pH 7 using 12 N HCl and diluted to 25 mL with 1 M sodium borate buffer (pH 9). Aliquots of these solutions were filtered through a 0.45 μm PVDF filters and then injected into a Nova-Pack C18 column (Waters, Mississauga, ON, Canada). An isocratic elution system consisting of 25 mM sodium acetate, 0.02% sodium azide (pH 9)/acetonitrile (91:9) delivered at 1 mL/min was used.

2.3 | Electrophoresis

The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of proteins was performed according to Laemmli et al. (1970) using a Minin-Protean 3 Gel Electrophoresis Unit (Bio-Rad) and Criterion TGX™ Precast Any kD gel (5671124 Bio-Rad Laboratories, Inc., CA, USA). About 2.5 mg of raw, extruded, cooked, and baked samples was dissolved in 1 mL Laemmli buffer (0.1 M Tris-Tricine, pH 6.8, 2% SDS, 5% β-mercaptoethanol, and 0.025% bromophenol blue), stirred for 1 h, boiled for 5 min, and then centrifuged at 10,000 *g* for 1 min and loaded onto the gel (20 μL samples, 20 μg protein/well) and run at 150 kV. Gel was stained using 0.125% Coomassie Brilliant Blue R-250 in 7% acetic acid and 40% MeOH (v/v) solution and stained in 7% acetic acid and 30% EtOH (v/v) solution. As a molecular marker, Precision Plus Protein™ standard (10–250 kDa, Bio-Rad Laboratories Inc., CA, USA) was used.

2.4 | Bioactive compounds

The quantification of saponins in the sample was performed following the method of Hiai et al. (1976), using diosgenin as a standard (0–0.125 mg/mL). Samples were mixed twice with methanol 80%, 200 μL of supernatants was pooled after centrifugation, and 250 μL of Vanillin and 2.5 mL of sulfuric acid were added. Absorbance was measured at 520 nm against distilled water as a blank. The content of saponins was reported as mg equivalents of diosgenin/g of sample. The content of phytates was determined following the method of Vaintraub and Lapteva (1988), using phytic acid as a standard (0–0.160 mg/mL). Samples (0.5 g) were mixed with 10 mL of 5% HCl, stirred for 1 h and then centrifuged (5000 rpm × 10 min). Subsequently, 200 μL of extract was diluted with 2.8 mL of distilled water and mixed with Wade reagent (1.0 mL). The absorbance was measured at 500 nm against distilled water as a blank. The phytate content was expressed as mg equivalents of phytic acid/g of sample. Finally, the content of total phenolics was determined in the raw and processed flours following the Folin–Ciocalteu method (Singleton et al., 1999), using gallic acid as a standard (0–100 mg/mL). Samples (0.5 g) were mixed with 10 mL of acidified methanol (1% HCl) and extracted for 18 h in dark conditions at room temperature. Afterwards, samples were centrifuged at 5000 rpm for 10 min. Supernatants (20 μL) were vortexed with distilled water (1.58 mL), Folin-Ciocalteau reagent (100 μL), and Na_2CO_3 10% (300 μL). Then, samples were kept away from light until reading; absorbance was measured at 760 nm against distilled water as a blank. Results were expressed as mg gallic acid equivalents per mg of sample (GAE mg/mg sample).

2.5 | In vitro protein digestibility-corrected amino acid score: Hydrolysates

Samples were digested following the method reported by Tinus et al. (2012), with few modifications. Briefly, the equivalent of 62.5 mg of protein was rehydrated in 10 mL of Milli-Q water, heated to 37°C and adjusted to pH 8.0. The samples were monitored for 10 min to record the stability of the pH, followed by the addition of a multienzyme cocktail containing trypsin (16 mg, 13,000–20,000 BAEE units/mg protein), chymotrypsin (31 mg, 40 units/mg protein) and protease (50–100 units/g solids). After the addition of the digestive cocktail, the subsequent pH drop was recorded for 10 min. Subsequently, the samples were transferred in a 95–99°C bath for 15 min and cooled down in an iced bath. Then the samples were centrifuged at 4°C, 6000 *g* for 30 min, and the supernatants were recovered. The *in vitro* protein digestibility (IVPD) was calculated as follows:

$$\text{IVPD}\,(\%) = 65.66 + 18.10 \times (\text{pH}_{0\text{min}} - \text{pH}_{10\text{min}})$$

Meanwhile the *in vitro* protein-digestibility corrected amino acid score (IVPDCAAS) was calculated as a product of the amino acid score (AAS) and IVPD% (Nosworthy et al., 2018).

2.6 | Nutritional parameters

AAS of raw and processed pulses' flours were calculated using the FAO/WHO/UNU (1985) reference pattern and using the following equation:

$$\text{AAS} = \frac{\text{mg of amino acids in 1 g total protein}}{\text{mg of amino acids in requirement pattern}} \times 100$$

Essential amino acid proportion on total amino acids (EAA/TAA) was calculated according to Pastor-Cavada et al. (2014) using the following equation:

$$\text{EAA/TAA}\,(\%) = \frac{\text{EAA}}{\text{TAA}} \times 100$$

Predicted biological value (BV) was calculated using the following equation (Pastor-Cavada et al., 2014):

$$\text{BV} = 10^{2.15} \times \text{Lys}^{0.41} \times (\text{Phe} + \text{Tyr})^{0.60} \times (\text{Met} + \text{Cys})^{0.77} \times \text{Thr}^{2.4} \times \text{Trp}^{0.21}$$

Protein efficiency ratio (PER) values were obtained from the amino acid composition of pulses samples based on the following five equations (Amza et al., 2013):

$$PER_1 = -0.684 + 0.456(Leu) - 0.047(Pro)$$

$$PER_2 = -0.468 + 0.454(Leu) - 0.105(Tyr)$$

$$PER_3 = -1.816 + 0.435(Met) + 0.780(Leu) + 0.211(His) - 0.944(Tyr)$$

$$PER_4 = 0.08084(Thr + Val + Met + Ile + Leu + Phe + Lys) - 0.1094$$

$$PER_5 = 0.06320(Thr + Val + Met + Ile + Leu + Phe + Lys + His + Arg + Tyr) - 0.1539$$

2.7 | Antioxidant potential of protein hydrolysates

Antioxidant potential of protein hydrolysates from raw, extruded, cooked, and baked pulses obtained from IVPD hydrolysis were evaluated with six different methods. Absorbance variations were monitored using a BioTeck™ microplate reader (BioTek Instruments, VT, USA) for DPPH scavenging activity, $Cu^{2+}$and Fe^{2+}chelating activity, reducing power, and β-carotene bleaching activity, while a BioTek™ fluorescent multimode microplate reader (Sinergy HTX, BioTek Instruments, VT, USA) was used for ORAC assay. Protein hydrolysate concentrations of 2.5, 5.0, and 10.0 mg/mL were assayed for all methods. Microplate readers were controlled by Gen5™ software (Fisher Scientific, Nepean, ON, Canada). To determine the concentration causing an inhibition of 50% (IC_{50}), standardized protein solutions of raw and processed pulse hydrolysates were prepared, and the data were fitted with the following equation:

$$y = a - b(x)$$

where *y* is the inhibition rate, *a* and *b* are the regression parameters, and *x* is the protein concentration (mg/mL). The IC_{50} value was obtained as follows:

$$IC_{50} = Ln\left[\frac{a-50}{b}\right]$$

2.7.1 | DPPH scavenging activity

DPPH radical scavenging activity was performed following the method proposed by Sánchez-Vioque et al. (2012), with some modifications. In a 96-well plate, 125 μL of 0.1 mM DPPH was added to 125 μL of raw and processed pulse hydrolysates. The plate was incubated for 30 min at room temperature with agitation and then read at 517 nm. The inhibition activity was calculated as follows:

$$\%\text{of inhibition} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

where A_0 is the absorbance of the negative control (distilled water) and A_1 is the absorbance of the samples.

2.7.2 | Metal chelating activities (Cu^{2+} and Fe^{2+})

The Cu^{2+} chelating activity was assessed following the method reported by Carrasco-Castilla et al. (2012a), with slight modifications. About 20.5 μL of raw and processed pulse hydrolysates was added into each well of a 96-well plate and mixed with 185 μL of sodium acetate buffer, 15 μL of copper solution (0.1968 mg/mL in 50 mM sodium acetate buffer, pH 6.0), and 9 μL of 2 mM pyrocatechol violet prepared in the same buffer. The plate was incubated 10 min at room temperature with agitation and read at 632 nm.

The Fe^{2+}chelating activity was assessed according to Carrasco-Castilla et al. (2012a), with slight modifications. A volume of 25 μL of each supernatant was added in a 96-well plate and mixed with 225 μL of 100 mM sodium acetate buffer at pH 4.9 and 30 μL of $FeCl_2 \bullet 4H_2O$ (0.036 mg/mL in sodium acetate buffer). About 12.5 μL of 40 mM Ferrozine solution prepared in the same buffer was added after incubation for 30 min at room temperature. Absorbance was measured at 562 nm.

Fe^{2+} and Cu^{2+} chelating activity was calculated as follows:

$$\%Chelating\ activity = \frac{ABS_{control} - ABS_{sample}}{ABS_{control}} \times 100$$

2.7.3 | Reducing power

The reducing power of supernatants was evaluated following the method reported by Sánchez-Vioque et al. (2012) and Carrasco-Castilla et al. (2012a). In each well, 50 μL of sample was added and mixed with 50 μL of 0.2 M phosphate buffer (pH 6.6) and 50 μL of 1% $K_3Fe(CN)_6$ and incubated at 50°C for 20 min. Then, 50 μL of 10% TCA and 10 μL of 0.1% $FeCl_3$ were added, and plates were incubated for another 10 min at 50°C with agitation. Absorbance was measured at 700 nm. Reducing power values ranged between 0.0 and 1.0.

2.7.4 | β-carotene bleaching activity

The β-carotene bleaching was measured according to Marco (1968), with some modifications. About 1 mL of β-carotene (2 mg/mL in chloroform) was mixed with 20 mg of linoleic acid and 100 mg of Tween-20®. Following chloroform evaporation under nitrogen, 200 μL of β-carotene solution was added to O_2-sparged water to reach an absorbance of 1.2 ± 0.1 at 450 nm. A volume of 50 μL of sample was mixed with 200 μL β-carotene solution and incubated in the dark at 50°C for 60 min before determination of absorbance at 450 nm in a microplate reader (BioTek Instruments, VT, USA). About 200 μL of β-carotene solution was mixed with 50 μL of distilled water (control). AA was calculated according to the following equation (Al-Saikhan et al., 1995):

$$DR = \frac{Ln\frac{ABS_{0min}}{ABS_{60min}}}{60}$$

where DR is the degradation rate among the absorbance at the beginning (ABS_{0min}) and the end of the reaction (ABS_{60min}) for control and samples. Using the DR, the antioxidant activity (AA) was calculated as % inhibition relative to the control as follows:

$$\%Antioxidant\ activity\ (AA) = \left(\frac{DR_{control} - DR_{sample}}{DR_{control}}\right) x\,100$$

where $DR_{control}$ is the degradation rate of β-carotene in the absence of sample. Results were expressed as percentage (%) AA.

2.7.5 | ORAC assay

The ORAC assay was performed as previously described (Huang et al., 2002). The declining fluorescein (0.08 μM) absorbance by AAPH• (150 mM) at 37°C was recorded at 5 min intervals over 60 min at 485/528 nm of excitation/emission. Potassium phosphate buffer (pH 7.4, 75 mM) was used to dissolve samples (2.5, 5.0 and 10.0 mg protein/mL) and Trolox standards (0–100 μM). The AOX was estimated using Trolox as the standard curve and the following equation:

$$AOX = \left[\frac{AUC_{sample} - AUC_{blank}}{AUC_{Trolox} - AUC_{blank}}\right]$$

where AUC_{sample} is the area under the curve of each sample, AUC_{blank} is the area under the curve of blank, and AUC_{Trolox} is the area under the Trolox curve.

2.8 | Statistical analyses

The experiments were carried out in triplicate. A one-way ANOVA with a Tukey's post-hoc test and Dunnett's test comparison analysis were used to observe the interactions among bioactive compounds and AOX, respectively, with a significance value of $p < 0.05$. Statistical version 12 (StatSoft, Tulsa, USA) was used to perform the statistical analyses. A principal component analysis (PCA) was performed for protein digestibility, DPPH, Cu^{2+} and Fe^{2+} chelation activity, reducing power, β-carotene bleaching activity, and ORAC using the software Statistica version 12 (StatSoft Power Solutions, Inc., Tulsa, OK, USA). All the components were analyzed.

3 | RESULTS AND DISCUSSION

3.1 | SDS-PAGE

The electrophoretic pattern of raw and processed pulses displayed multiple bands ranging from ~10 to 105 kDa (Figure 2). For RL, bands at 70 kDa (associated to convicilin subunits) were still present in all the processed samples, but they were less intense in the baked sample than in the extruded and cooked samples. A set of bands at 40–50 kDa (associated to vicilin subunits) was of similar intensity for the extruded sample compared to the raw sample; they were slightly less intense in the cooked sample but dramatically less intense for the baked sample. Similar results were observed for a set of bands at 28–35 kDa (associated to α-subunits of legumin); bands at 12–18 kDa (associated to γ-vicilin or albumin subunits) were slightly intensified in cooked and baked samples, while the extruded sample showed the least intensity. In GL, similar band patterns were observed as for RL but with slight differences. The intensity of a set of bands at 40–50 kDa was more intense for the cooked and baked GL samples than for the corresponding RL samples, while being still less intense than for the raw and extruded GL samples. A similar pattern was observed for the bands detected at 28–35 kDa. For the set of bands at 12–18 kDa (associated to γ-vicilin), the intensity of the baked sample was more intense than for the corresponding RL sample.

GSP showed bands at 50 kDa (associated to α-subunits of legumin) and at 30 kDa (associated to β-legumin subunits) that were slightly less intense after processing; a set of 10–12 kDa (associated to albumin subunits) had a decreased intensity in cooked and baked flours. In YSP, bands at 75 kDa showed a slight decrease with processing, especially for the cooked and baked samples; a similar pattern was observed for the 50 kDa bands. Bands at 35–37 kDa (associated to lectins) had a high intensity in extruded and cooked samples but were slightly less intense in baked flour. For the 30 kDa bands, the extruded sample showed similar intensity as for the raw sample, while the cooked and baked flours had bands of lesser intensity. And lastly, 10–12 kDa bands showed less intensity for the processed samples than for the raw sample, especially for the cooked and the baked flours.

In CC, bands at 95 kDa (associated to β-subunits of conglycinin) were more intense in raw samples than for the processed samples. For the bands at 72–75 kDa (associated to α′- or α-subunits of conglycinin), the bands were barely affected in the extruded sample, but bands were less intense in the cooked and baked samples. Bands at 45–50 kDa (associated to basic β-subunits of glycinin or β-subunits of conglycinin) were less intense after processing, especially for the cooked and baked flours. The set of bands at 30–35 kDa (associated to basic β-subunits of glycinin) and bands at 18–20 kDa (associated to acid α-subunits of glycinin) were barely affected by processing.

In FB, bands at 100–105 kDa (associated to legumin) showed a decrease in intensity for all the processed samples. Other sets of bands at 55–70 kDa (associated to phaseolin) were about the same intensity in cooked samples but decreased in extruded and baked flours. A band at 35 kDa (associated to α-type phaseolin) was not affected by processing, except for the extruded sample, which was less intense. Another set of bands at 15–20 kDa (associated to β-type phaseolin or phytohemagglutinin) was barely affected, except for the extruded samples, which were less intense. RKB displayed bands at 109–115 kDa (associated to legumin), which were less intense in processed flours. A set of bands at 37–57 kDa (associated to α-type phaseolin) was less intense after processing, especially for the extruded sample. In BB, bands at 51–55 kDa (associated to phaseolin) were less intense in processed flours than in the raw sample, especially for the extruded and the baked samples; a similar pattern was observed for the bands at

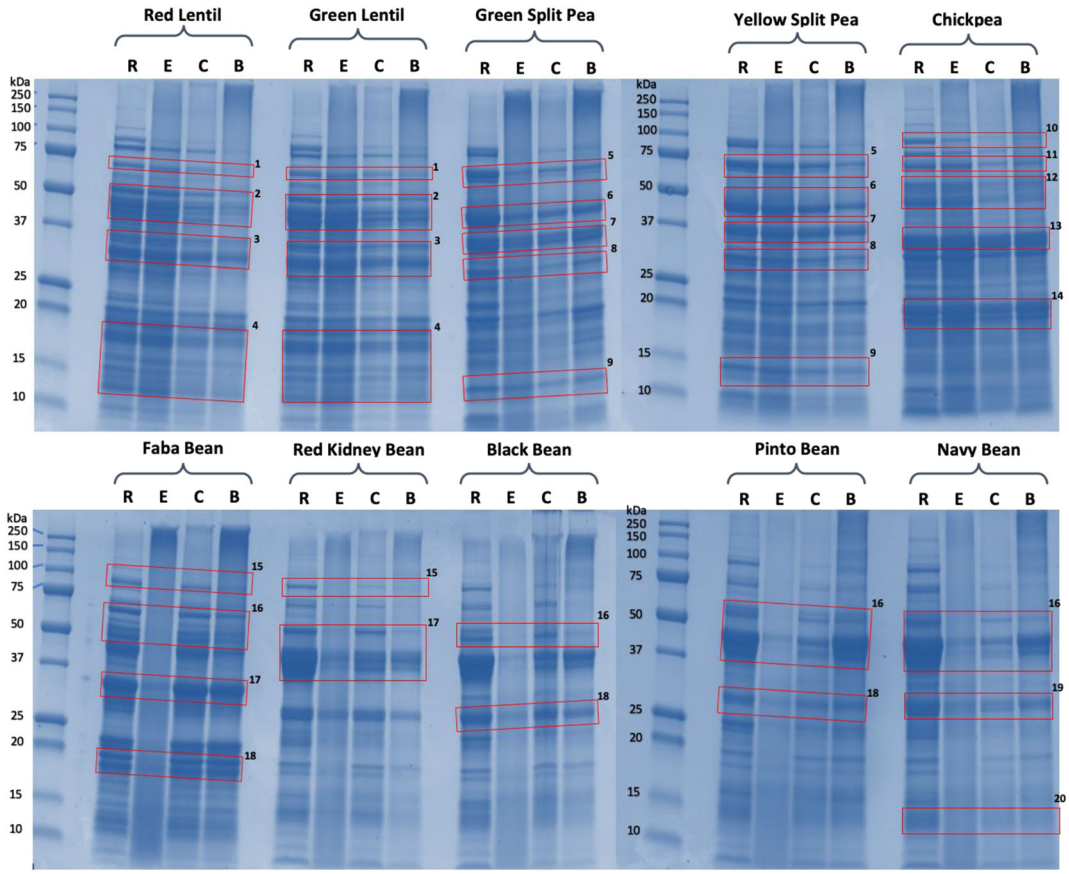

FIGURE 2 SDS-PAGE of pulse flours (under reducing conditions). Proteins: **1.** Convicilin; **2.** Vicilin; **3.** Legumin (α-subunit); **4.** γ-vicilin or albumin subunits; **5.** 75 kDa protein; **6.** Legumin (α-subunit); **7.** Lectins; **8.** Legumin (β-subunit); **9.** Albumin (subunits); **10.** Conglycinin; **11.** Conglycinin (α'- or α-subunit); **12.** Basic β-subunits of glycinin/β subunits of conglycinin; **13.** Glycinin (β-subunit); **14.** Glycinin (α-subunint); **15.** Legumin; **16.** Phaseolin; **17.** α-type phaseolin; **18.** β-type phaseolin or phytohemagglutinin; **19.** α-type phaseolin or erythroagglutinating phytohemagglutinin; **20.** β–subunit of α-amylase inhibitor

31 kDa (associated to α-type phaseolin). In PB, the main set of bands was observed at 53–69 kDa (associated to phaseolin), as well as bands of 30 kDa (associated to α-type phaseolin), whose intensity was decreased with processing, except for the baked sample, which was barely affected. In NB, a band at 51 kDa (associated to phaseolin) had a strong intensity, which decreased in processed flours. Bands at 31 kDa (associated to α-type phaseolin or erythroagglutinating phytohemagglutinin) were less intense after processing, especially after extrusion. Bands at 12 kDa (associated to β-subunit of α-amylase inhibitor) were slightly less intense after processing.

Thermal processing is the most important treatment employed to enhance nutritional properties and functionality of proteins from pulses (Alfaro-Diaz et al., 2021; Sahni et al., 2020). Some heat-processed pulses have shown degradation particularly in the high and low molecular weight sections of the banding pattern, which is represented by less bands and bands of lesser intensity. In some cases, the loss of bands could indicate polypeptide aggregation induced by heating and not necessarily protein denaturation or degradation (Avila Ruiz et al., 2016). For cooked samples, some proteins such as albumins (water soluble) may also have been lost in the soaking, cooking, and rinsing water (Habiba, 2002; Pasqualone et al., 2020).

3.2 | Bioactive compounds of raw and processed pulses

3.2.1 | Saponins

Saponin content of raw and heat-treated pulses is shown in Figure 3a. Saponin content in raw pulses ranged between 143.89 and 271.91 mg of saponin equivalents/mg dw (RL < FB < YSP < GSP < CC < GL < RKB < NB < BB < PB). The impact of the extrusion process on the saponin content did not show a clear pattern, since the saponin content was increased, decreased, or not affected, depending on the pulses. Saponin content decreased by 17% and 13% in extruded GSP and PB, respectively. Baking did not statistically ($p > 0.05$) reduce the saponin content for any sample. In fact, for some pulses, the saponin content after baking was higher than for the corresponding raw sample. Meanwhile, cooking successfully decreased ($p < 0.05$) the saponin content in RL, GL, YSP, CC, RKB, BB, PB, and NV (27%, 28%, 44%, 12%, 22%, 31%, 30%, and 25%, respectively). Saponins occur in legumes as oligopolymers and are protective agents against insects (Chaieb, 2010). Saponins are thermal sensitive and water soluble. Therefore, during food

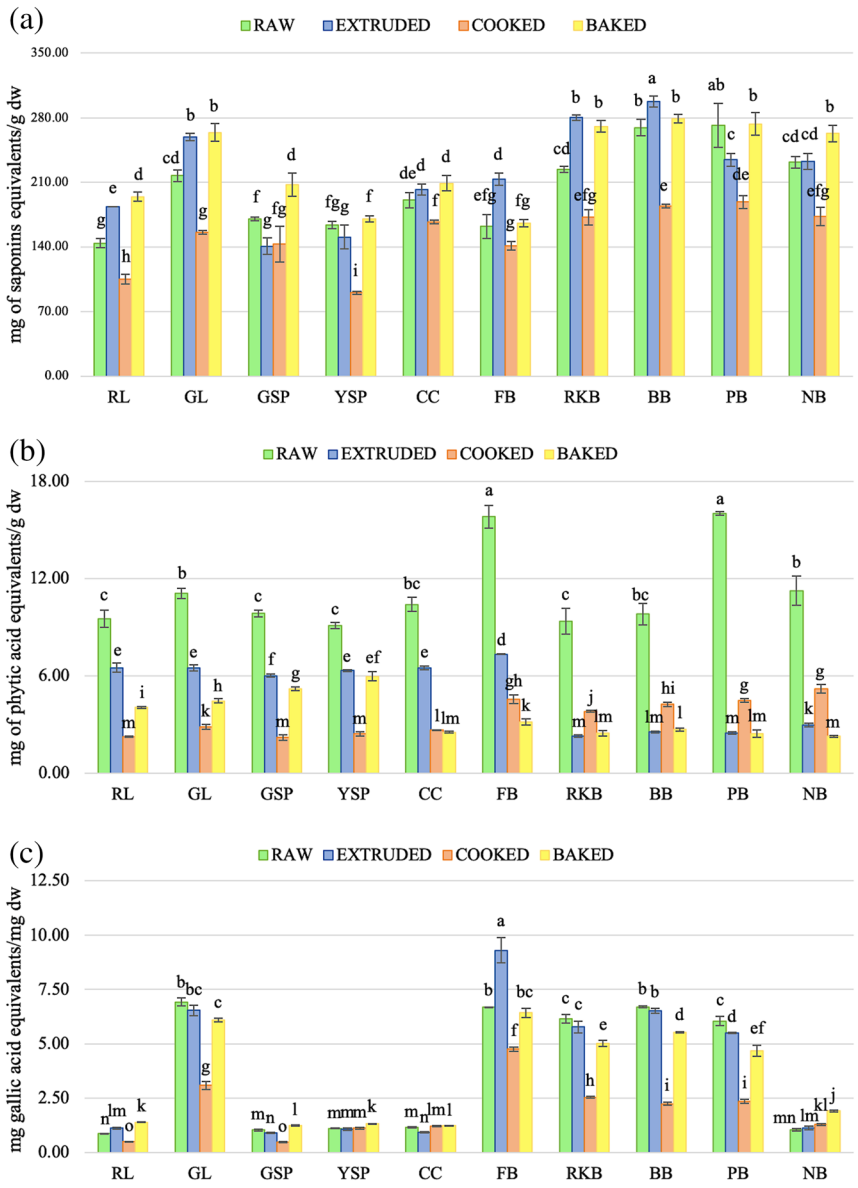

FIGURE 3 Bioactive compounds evaluated on selected raw and heat-processed pulses. (a) Saponins content in raw and processed pulse flours (mg equivalent of saponin/100 g dw); (b) phytic acid content in raw and processed pulse flours (mg equivalent of phytic acid/100 g dw); (c) total phenolic compounds content in raw and processed pulse flours (mg equivalent of gallic acid/100 g dw). Same letters indicate nonstatistical differences ($p < 0.05$) between samples

processing, such as soaking and cooking, these can migrate and/or solubilize into the soaking water. Additionally, presoaking may facilitate saponin release during heating (Shi et al., 2004). Usually, extrusion and baking do not include hydrothermal steps, and saponins are resistant to these dry-heating conditions (Barakat et al., 2015; Knudsen et al., 2006). Shi et al. (2004) reported a loss of saponins in chickpeas, faba bean, and pigeon pea after soaking and cooking (>52% loss). They attributed this effect to the leaching of saponins by diffusion in the soaking water.

3.2.2 | Phytic acid

Figure 3b shows the results of phytic acid content in raw and processed pulses. Phytic acid content is higher in all raw samples, with values ranging between 9.10 and 16.01 mg of phytic acid equivalents/g dw (YSP < RKB < RL < BB < GSP < CC < GL < NB < PB < FB). All the processing methods ($p < 0.05$) reduced the phytic acid content of the samples, but each process' effectiveness depended on the pulse variety. Extruded RL and YSP showed a decrease of 30%, but extruded RKB, BB, PB, and NB presented a more pronounced effect, with decreases of 75%, 74%, 84%, and 73%, respectively. In all cooked samples, phytates were dramatically decreased, especially in RL, GL, GSP, YSP, CC, FB, and BB, where this was >70%. Baking decreased the phytic acid content in lentils and split peas by 34%–60%, respectively, but chickpeas and beans showed a reduction higher than >72%. The phytic acid present in beans seems to be more thermosensitive than saponins, since its content was decreased by at least 53% in our five beans after processing, with the highest effect for those subjected to dry heat treatments.

The phytic acid content of raw pulses reported in this study is within the range reported by Gupta et al. (2013) for some other legume seeds (2.2–23.2 mg phytic acid/g dw). El-Adawy et al. (2000) observed that the reduction in phytic acid content during the cooking

process occurred during the soaking step by leaching (depending on the nature of the phytates) and due to the increase of phytase activity induced by the soaking water. This is in agreement with our results. Furthermore, Habiba (2002) attributed the decrease of phytates during traditional cooking (boiling water) to their binding with other nutrients such as proteins and minerals, limiting their bioavailability and detection by spectrophotometric methods. Patterson et al. (2016) and Rathod and Annapure (2016) reported a loss of phytic acid that was more or less significant depending on the extrusion parameters used (moisture, pressure and temperature) in different pulses. This reduction may be due to thermal degradation and/or a reactive chemical change and/or insoluble complex formation and could be enhanced by the increase of moisture in the extruder (Rathod & Annapure, 2016). Patterson et al. (2016) also observed a reduction in phytic acid after roasting, which could suggest that the baking process in our study might have the same effect. Champ (2002) explained that the typical process used to reduce phytic acid content in seeds is enzymatic degradation (phytase), germination, and fermentation, but in this study extrusion, soaking + cooking and baking processes showed interesting results for developing pulse food products with low phytic acid content.

3.2.3 | Polyphenols

The phenolics content is shown in Figure 3c. Phenolic compounds in raw samples showed a high variability, ranging between 0.87 and 6.93 mg of gallic acid equivalents/g dw (RL < GSP < NB < YSP < CC < PB < RKB < FB < BB < GL). Extrusion only resulted in a reduction ($p < 0.05$) of phenolic compounds in GSP, CC, and PB of 11%, 21%, and 9%, respectively. Meanwhile, cooking resulted in a decrease ($p < 0.05$) in lentils, GSP, FB, RKB, BB, and PB of between 28% and 66%. Baking showed a reduction ($p < 0.05$) of 12%, 19%, 17%, and 22% in GL, RKB, BB, and PB, respectively. As was observed for phytic acid, soaking/cooking resulted in the highest decrease of phenolic compounds for most of the pulses in this study.

Phenolics are mainly located in the seed coats of pulses, since they act as the first layer of protection. Therefore, phenolic concentration and diversity vary due to a wide variety of factors such as environmental conditions and varietal traits (Singh et al., 2007). Food processing methods may modify the profile of phenolic compounds in pulses; as an example, germination and moisture treatments successfully enhanced their degradation (Khandelwal et al., 2010). This same study highlighted the varietal effect on tannin content, showing that colored seeds contain a higher tannin content, which is also observed with the phenolic content presented in this research. In addition, as observed for phytic acid, soaking seeds before cooking is known to decrease the polyphenol content, not only by leaching into the soaking water but also by activating polyphenol oxidase, which degrades the polyphenols, leading to their destruction (Khandelwal et al., 2010). Ordinary cooking treatment as described by Habiba (2002) also showed an effect on the loss of some phenolics.

Ragaee et al. (2014) observed that an increase of phenolic compounds could be observed in some samples after thermal processing produced browning reactions, as was the case with extrusion (Brennan et al., 2011). This is due to the dissociation of conjugated phenolics and to the polymerization and/or oxidation reactions and the formation of new phenolics. In our work, the increase of phenolics was particularly significant in FB after extrusion, but for most of the other samples, a slight increase or decrease was observed. Additionally, Ragaee et al. (2014) noticed both effects—an increase or decrease of phenolics—after roasting, which is the process closest to baking. Additionally, phenolics from germinated or microwave-roasted black chickpea, as well as solid-state fermented lentils, showed a significant increase after these processing methods were applied (Dhull, Punia, Kidwai, et al., 2020; Kumar et al., 2020). Our results showed an increase of phenolics after baking for the samples containing a low amount of phenolics in the raw samples (RL, GSP, YSP, CC, and NB), whereas a decrease was observed in those with a high phenolic content in the raw samples (GL, RKB, BB, and PB). Champ (2002) mentioned that mechanical processes such as dehulling are commonly applied to reduce phenolic compounds from outer layers. This study showed interesting results for soaking/cooking processed samples, particularly for those presenting the highest phenolic content, but adding a dehulling pretreatment should help to considerably reduce phenolic compounds before other processing steps are applied.

3.3 | In vitro protein digestibility

IVPD of processed and raw pulses is presented in Table 1. Raw *Phaseolus vulgaris* beans showed between 75% and 78% protein digestibility, followed by CC, FB, lentils, and split peas with 79%, 82%, 83% to 84%, and 84% to 85%, respectively. After processing, all samples showed slight ($p < 0.05$) IVPD enhancement. Extrusion showed an increase of IVPD that ranged between 2.5% and 9.5%, while the increase observed for cooked samples was between 3.5% and 10.7% and between 2.2% and 8.4% for baked samples. Different studies observed an increase in protein digestibility after the application of various processing methods. Habiba (2002) and El-Adawy et al. (2000) observed an increase in digestibility after traditional cooking, microwave cooking, pressure cooking (autoclaving), and soaking alone. Protein digestibility was improved after cooking, not only due to the reduction of phytic acid and tannin content but also as discussed previously by Habiba (2002), due to the heating effect on the tertiary structure of the proteins, which allows better enzyme accessibility. El-Adawy et al. (2000) suggested that soaking activates proteases, which hydrolyze high molecular weight proteins into low molecular weight subunits, changing their conformation, which could enhance proteolysis during gastrointestinal digestion. Furthermore, Rathod and Annapure (2016) also found an increase of protein digestibility after extrusion, supporting the idea that the reduction in the content of some bioactive compounds, which can interfere with protein digestion, can have a positive effect on protein digestibility.

TABLE 1 Nutritional parameters of selected raw and heat-processed pulses' flours

Pulses	HT	IVPD (%)	AAS	EAA/TAA (%)	BV	PER_1	PER_2	PER_3	PER_4	PER_5	IVPDCAAS
Red lentil (RL)	**R**	84.24	135.73	47.13	35.22	2.96	2.89	2.36	2.84	2.97	62.07
	E	90.64	123.84	43.87	49.51	2.68	2.58	1.81	2.52	2.78	73.19
	C	87.50	134.78	47.46	69.91	3.35	3.22	2.55	2.79	3.03	64.92
	B	88.10	102.88	36.51	13.80	2.41	2.37	1.50	2.09	2.28	85.64
Green lentil (GL)	**R**	83.28	143.22	51.23	45.47	3.23	3.14	2.63	3.10	3.25	58.15
	E	87.20	122.30	44.26	32.67	2.73	2.65	1.88	2.56	2.80	71.30
	C	87.02	131.80	46.46	62.59	3.16	3.04	2.34	2.74	2.95	66.02
	B	86.54	99.26	35.18	13.99	2.14	2.13	1.41	2.03	2.18	87.18
Green split pea (GSP)	**R**	84.85	142.52	46.65	51.04	2.79	2.70	1.96	2.72	2.93	59.53
	E	88.53	97.26	35.58	16.79	2.01	1.97	0.89	2.00	2.22	91.02
	C	88.71	157.13	45.09	52.11	2.92	2.81	1.92	2.57	2.80	56.46
	B	88.10	140.48	43.11	57.46	3.91	3.80	3.73	2.41	2.67	62.72
Yellow split pea (YSP)	**R**	85.09	149.88	48.64	48.91	2.92	2.80	1.70	2.87	3.08	56.77
	E	88.65	103.85	38.00	20.84	2.19	2.12	0.94	2.15	2.40	85.36
	C	88.77	130.78	45.91	39.25	3.06	2.92	1.89	2.70	2.93	67.87
	B	87.68	103.24	35.93	15.86	2.20	2.14	0.93	2.04	2.25	84.93
Chickpea (CC)	**R**	78.93	135.72	44.62	37.44	2.57	2.53	2.12	2.57	2.77	58.16
	E	86.54	153.77	45.68	62.28	2.69	2.66	2.34	2.56	2.80	56.27
	C	84.79	133.01	45.43	58.16	2.94	2.87	2.30	2.58	2.85	63.74
	B	85.87	117.82	40.52	30.86	2.59	2.56	2.04	2.28	2.52	72.88
Faba bean (FB)	**R**	81.77	154.90	52.43	67.57	3.25	3.08	2.14	2.93	3.34	52.79
	E	83.16	113.87	41.91	25.75	2.69	2.58	1.58	2.25	2.66	73.03
	C	85.09	131.22	48.68	43.70	3.48	3.34	2.72	2.80	3.12	64.84
	B	84.00	96.46	34.94	74.16	1.98	1.96	1.06	1.92	2.17	87.09
Black bean (BB)	**R**	75.01	170.56	52.26	106.87	3.36	3.23	2.76	3.28	3.30	43.98
	E	82.92	165.31	46.15	115.84	3.02	2.88	2.04	2.76	2.88	50.16
	C	84.06	168.34	51.02	107.41	3.71	3.55	3.07	3.25	3.24	49.94
	B	82.13	120.57	37.83	49.28	2.36	2.32	1.51	2.32	2.35	68.12
Red kidney bean (RKB)	**R**	75.13	150.18	47.54	68.16	3.08	2.97	2.38	3.01	3.01	50.03
	E	81.71	140.27	43.19	70.95	2.83	2.72	1.97	2.59	2.72	58.25
	C	84.42	132.26	40.60	27.42	2.46	2.43	1.93	2.54	2.51	63.83
	B	80.02	111.57	34.26	126.28	1.95	1.96	1.32	2.11	2.08	71.72
Pinto bean (PB)	**R**	77.79	157.25	48.07	63.32	2.98	2.86	2.16	3.02	3.04	49.47
	E	81.41	137.56	43.62	82.07	2.87	2.73	1.87	2.62	2.77	59.18
	C	84.67	142.15	44.05	51.98	3.22	3.10	2.47	2.74	2.79	59.56
	B	79.66	127.57	38.76	26.69	2.38	2.33	1.62	2.36	2.39	62.44
Navy bean (NB)	**R**	78.15	159.50	48.88	92.31	3.31	3.22	2.98	3.06	3.07	49.00
	E	86.96	142.44	45.30	84.78	3.04	2.91	2.12	2.73	2.87	61.05
	C	84.60	152.79	46.30	73.00	3.27	3.15	2.59	2.90	2.92	55.37
	B	82.73	123.10	38.45	33.03	2.35	2.31	1.65	2.37	2.38	67.21

Abbreviations: AAS, amino acid score; B, baked; BV, biological value; C, cooked; E, extruded; EAA/TAA, essential amino acid/total amino acid; HT, heat treatment; IVPDCAAS, protein digestibility corrected amino acid score; PER, protein efficiency ratio; R, raw.

These authors also reported a higher protein digestibility when extrusion was used, in comparison with other processing methods, such as ordinary cooking and microwave cooking. This was also observed in this study but only for lentil, chickpea, and one bean variety (NB). Shimelis and Rakshit (2007) obtained an increase of protein digestibility for kidney beans, as we did, after several processing techniques such as hydration, cooking, soaking + cooking, autoclaving, and germination.

3.4 | Nutritional parameters

The calculated nutritional parameters are presented in Table 1. The AAS, IVPD and amino acid profile (Supplementary material 1) are used to determine if a protein is complete, in combination with other protein quality methods such as nitrogen balance (NB), PER, estimated PER and maximum PER, net protein ratio (or retention) (NPR), protein rating (PR), net protein utilization (NPU), BV (apparent, true, and relative), and the PDCAAS. AAS of raw, extruded, cooked, and baked pulses flours ranged between 135.72–170.56, 103.85–165.31, 130.78–168.34, and 96.46–140.48, respectively. In general, AAS was lower for the baked samples than for the raw, extruded, and cooked samples. AAS of raw and cooked samples is higher than that of four flours from tribe *Fabae* (AAS 117.67–123.03) (Pastor-Cavada et al., 2014) and a faba bean protein hydrolysate (AAS 119.31) (Parya Samaei et al., 2020), as well as several processed beans (AAS 61–92) (Nosworthy et al., 2018). The essential amino acid proportion (% EAA/TAA) of raw, extruded, cooked, and baked flours were 44.62%–52.43%, 35.58%–46.15%, 40.60%–51.02%, and 34.26%–43.11%, respectively. Baked pulses had the lowest EAA proportion, while raw flours presented the highest values in most cases. These changes in the amino acids profile, might be due to the negative impact of heat treatment on the amino acid's bioavailability. As an example, lysine can participate in Maillard reactions with reducing sugars or other aldehyde compounds throughout heat processing (Gilani & Sepehr, 2003). Moreover, during heat processing and/or alkaline extraction, racemization of L-amino acids and the formation of crosslinked peptide chains such as lysinoalanine are prone to be produced, resulting in a loss of lysine, cysteine, and threonine, thus reducing protein digestibility and having a negative impact on protein quality parameters (Gilani & Sepehr, 2003; Sarwar, 1997). Pastor-Cavada et al. (2014) reported an EAA/TAA of 39.90%–41.75% in raw *Fabae* pulses, which are lower than our findings.

BV of samples were 35.22–106.87, 16.79–115.84, 27.42–107.41, and 13.80–76.28, respectively, for raw, extruded, cooked, and baked pulses. Protein sources with BV higher than 70% are considered as proteins of good nutritional quality (Amza et al., 2013). BV of proteins is based on the content of seven amino acids, in which methionine and cysteine (sulfur amino acids), as well as tryptophan (EAA), are the first and second limiting amino acids in pulses, thus having a negative impact on the calculation of this value. Despite this, raw BB and NB, as well as extruded BB, RKB, and NB, cooked BB and NB, and baked FB and RKB showed a BV > 70.95%, while the lowest BV was found in baked lentils (<14.00). A protein hydrolysate from faba bean had a BV of 88.01% (Parya Samaei et al., 2020), being higher than what was observed in this study for raw and processed FB flours, but lower than raw and extruded BB, RKB, and NB, as well as cooked BB and baked RKB. Pastor-Cavada et al. (2014) reported BV ranging from 26.40%–38.41% in some *Fabae* pulses (belonging to the genera *Lathyrus*, *Lens*, *Pisum* and *Vicia*), only baked PB and NB, and cooked RKB fall within this range. BV provides a measure of how well the absorbed amino acid profile matches the requirement (FAO/WHO/UNU, 1985). All protein quality evaluation methods have *pros* and *cons*; as an example a protein that lacks one indispensable amino acid can still present a BV of up to 40%, and it also does not take into consideration the importance of other factors that can influence protein digestion (negatively and/or positively) and interaction of proteins with other dietary factors before absorption, such as binding of tannins to proteins (reducing protein digestibility), non-enzymatic browning (Maillard reaction) between reducing sugars from starch hydrolysis and proteins (FAO/WHO/UNU, 1985; Khattab et al., 2009).

The theoretical PER based on different amino acid profiles showed the following results. PER_1 showed values of 2.57–3.36, 2.01–3.04, 2.46–3.48, and 1.95–3.91 for raw, extruded, cooked, and baked pulses flours, respectively. Higher PER_1 values were found in cooked samples, with a notable exception in baked GSP, since most of baked samples showed the lowest PER_1. PER_2 and PER_3 presented the same trend than for PER_1, but the values for raw, extruded, cooked, and baked samples ranged between 2.53–3.23, 1.97–2.91, 2.43–3.55, and 1.96–3.80 for PER_2, while the values for PER_3 varied between 1.70–2.98, 0.89–2.34, 1.89–3.07, and 0.93–3.73, respectively. PER_4 and PER_5 also showed similar behavior with values of 2.57–3.28, 2.00–2.76, 2.54–3.25, and 1.92–2.41 for raw, extruded, cooked, and baked pulses, respectively, for PER_4 and 2.77–3.34, 2.22–2.88, 2.51–3.24, and 2.08–2.67 in raw, extruded, cooked, and baked samples for PER_5. Higher PER_4 and PER_5 values were found in raw GL, YSP, and the five bean cultivars, while the lowest values were again identified in baked flours. Most plant based-protein foods have a PER of 1.2–2.4, while animal proteins have PER values of 3.1–3.7 (Mariotti, 2017). Nosworthy et al. (2018) reported PER values of 0.43–1.52 in five processed bean genotypes; nevertheless, this measurement was carried over a 4-week feeding trial (in vivo assay), thus measuring directly how the protein source impacts growth, therefore, showing differences with the theoretical values obtained in this study. Furthermore, higher values of 3.07 for PER_1-PER_3 were observed in *Fabae* pulses by Pastor-Cavada et al. (2014), being lower than those obtained for raw and extruded pulses flours in this research. Methionine is the only limiting amino acid in pulses considered for PER_3-PER_5 calculation, which has a negative influence in PER values for most of cases, especially on baked GSP and raw and/or extruded beans, in which this sulfur amino acid is particularly low. It has been suggested that the use of high temperatures and shear pressures used during extrusion and/or baking can denature and/or degrade proteins and certain amino acids, thus having a direct impact on protein quality (Frias et al., 2011).

Finally, the higher protein digestibility corrected amino acid scores (IVPDCAAS) were found in baked (IVPDCAAS 62.49–87.18) and extruded (IVPDCAAS 50.16–91.02) samples, although the lowest IVPDCAAS values were found in most cases for raw pulses (IVPDCAAS 43.98–62.07). In raw *Fabae* pulses, Pastor-Cavada et al. (2014) reported IVPDCAAS between 32 and 47, which are similar to the values observed for the raw samples in this study, but lower than for all processed pulses. Additionally, bean cultivars fall within the results obtained by Nosworthy et al. (2018), (IVPDCAAS 49.81–70.19) for five processed beans. Furthermore,

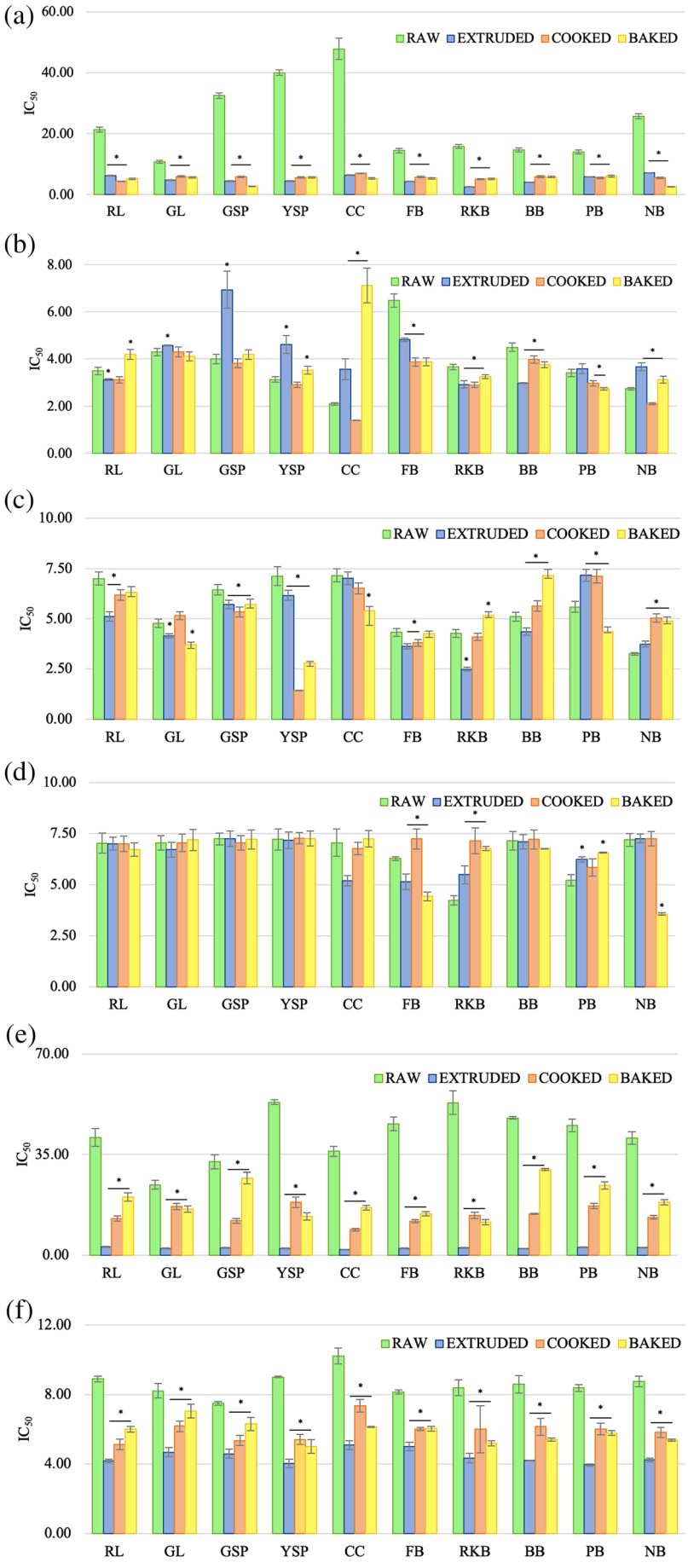

FIGURE 4 Half-maximal inhibitory concentration (IC_{50}) of raw and processed pulses on selected bioactivity tests. (a) DPPH scavenging activity; (b) Cu^{2+}chelating activity; (c) Fe^{2+}chelating activity; (d) reducing power; (e) β-carotene bleaching activity; (f) oxygen radical antioxidant activity (ORAC) assay. RL (red lentil); GL (green lentil); GSP (green split pea); YSP (yellow split pea); CC (chickpea); FB (faba bean); RKB (red kidney bean); BB (black bean); PB (pinto bean); NB (navy bean). Asterisks (*) indicate statistical differences ($p < 0.05$) between raw and heat-processed samples

Nosworthy et al. (2018) found a positive correlation between the IVPDCAAS and true protein digestibility, thus suggesting that this could be used as an alternative for in vivo evaluation of pulse protein ingredients, being less expensive, time-consuming, easy to use, and showing a general pattern for raw and processed pulses.

3.5 | Antioxidant potential of protein hydrolysates

The antioxidant capacity (AOX) evaluated in raw and processed pulse flours is summarized in **Supplementary material 2**. For practical purposes, the AOX is presented as the half-maximal inhibitory concentration (IC_{50}), which is the concentration required to result in a 50% AOX (Alam et al., 2013).

3.5.1 | DPPH

The 50% inhibitory concentration (IC_{50}) of the DPPH radical (Figure 4a) was calculated from DPPH scavenging results. All processed pulses showed statistically lower ($p < 0.05$) IC_{50} values when compared to those of raw samples. Raw CC presented an IC_{50} = 47.77 mg/mL, making it the least effective DPPH scavenger raw sample. Nevertheless, after processing, the extruded, cooked, and baked CC showed lower IC_{50} values of 6.43, 7.01, and 5.34 mg/mL, respectively. For RL, the highest decrease of IC_{50} was calculated for cooked RL (>79%), while in GL, the best IC_{50} was found in the extruded sample (>56%). Drastic changes for IC_{50} values were observed in GSP and YSP, since the IC_{50} in raw samples were 32.53 and 40.00 mg/mL, respectively. However, for processed samples IC_{50} ranged between 5.91 and 2.77 mg/mL in GSP and between 5.69 and 4.49 mg/mL in YSP; thus, an increase of DPPH scavenging capacity of between 85.4% and 91.5% and between 85% and 88%, respectively, was observed. Similar results were obtained for NB, since the raw NB exhibited an IC_{50} = 25.79 mg/mL and processed NB samples varied from IC_{50} = 7.19–2.62 mg/mL; therefore, the scavenging capacity improved by between 72% and 90%. Other beans also showed enhanced DPPH scavenging activity after extrusion, cooking or baking processing, with values ranging between 53% and 83%.

It is known that processing methods can enhance the antioxidant properties and other health beneficial properties in pulses (Dhull, Punia, Kidwai, et al., 2020; Dhull, Punia, Sandhu, et al., 2020). Extruded powder of lentil-orange peel at 130°C and between 12% and 20% of feed moisture showed DPPH scavenging activity over 93.6% compared to non-extruded orange-lentil peel powder (Rathod & Annapure, 2016). Morales et al. (2015) and Amarowicz et al. (2009) found that the DPPH scavenging activity of pulse flours increased >90% with extrusion and the addition of some other food ingredients. Contrary to the results published by Lv et al. (2018), our results suggest that the extrusion (with slow screw speed) and cooking processing enhanced the DPPH scavenging activity in lentils.

3.5.2 | Metal chelating properties

Compared to DPPH scavenging capacity, the Cu^{2+} chelating activity did not show a clear tendency among raw and processed samples (Figure 4b). The IC_{50} of raw RL (3.50 mg/mL) was statistically decreased ($p < 0.05$) after extrusion and cooking (3.12 mg/mL for both). In GL and GSP, the treatments enhanced ($p < 0.05$) the Cu^{2+} activity, except in extruded samples, where the IC_{50} showed a significant ($p < 0.05$) increase of >6% and >42%, respectively. A similar behavior was found in YSP and CC, since not only did extruded samples show less Cu^{2+} chelating activity but also baking process showed no improvement. Also, extruded and baked YSP samples presented an IC_{50} increase of approximately 32% and 11%, respectively, and 40% and 70% for extruded and baked CC, respectively. However, the Cu^{2+}chelating activity was improved for almost all processed beans. Raw FB showed an IC_{50} = 6.48 mg/mL; a decrease ($p < 0.05$) of 25% was observed for extruded FB (IC_{50} = 4.82 mg/mL), and a decrease of 40% was observed for cooked and baked FB (IC_{50} = 3.87 and 3.88 mg/mL, respectively). For RKB, cooking showed the highest Cu^{2+} chelating improvement (IC_{50} = 2.90 mg/mL, ∼20%). A similar value was observed in baked PB (IC_{50} = 2.74 mg/mL), compared to the raw PB sample (IC_{50} = 3.41 mg/mL). However, for BB, the extrusion process enhanced ($p < 0.05$) the Cu^{2+} chelating activity up to 44%. Finally, NB only showed an enhancement ($p < 0.05$) for the baked sample, with an IC_{50} = 2.11 mg/mL, which is 23% lower than for raw NB (IC_{50} = 2.74 mg/mL).

The Fe^{2+}chelating activity is shown in Figure 4c. The IC_{50} ($p < 0.05$) was decreased by 11% in extruded RL (IC_{50} = 5.12 mg/mL) and 26% for cooked RL (IC_{50} = 6.19 mg/mL), in comparison to raw RL (IC_{50} = 7.00 mg/mL). Meanwhile, extruded GL showed an IC_{50} = 4.15 mg/mL and an IC_{50} = 3.70 mg/mL for baked GL, which represents a decrease of 13% and 22%, respectively, when compared to the raw GL (IC_{50} = 4.78 mg/mL). GSP and YSP showed a decrease in IC_{50} ($p < 0.05$) by about 11% to 17% and 13% to 80%, respectively, after any heat processing applied. A decrease in IC_{50} of around 9% and 24% for cooked and baked CC, respectively, was observed against the raw CC. In FB and RKB, the antioxidant capacity of extruded and cooked samples was improved by 12% to 16% in FB and 4% to 41% in RKB. The extruded BB and the baked PB showed a decrease in IC_{50} by about 14% (IC_{50} = 4.37 mg/mL) and 22% (IC_{50} = 4.36 mg/mL), respectively, when compared to the corresponding raw samples. Statistically significant ($p < 0.05$) improvements of Fe^{2+}chelating activity were detected for processed NB.

Chelation activity of transition metals is related to antioxidant effects, since metal ions promote oxidative damage in the human body, due to Fenton reactions (Saiga et al., 2003). Cu^{2+} chelating activity seems to be somewhat higher than Fe^{2+}. These results are in agreement with those observed by Carrasco-Castilla et al. (2012b), who evaluated the antioxidant and chelating properties of protein hydrolysates from bean protein fractions. Another reduction in Fe^{2+} chelating activity was reported by Jamdar et al. (2017) on digested soaked-cooked chickpea, being <50% compared to dried samples. This effect could be due to the soaking step carried out before cooking and IVPD.

3.5.3 | Reducing power

The reducing power of selected pulses is shown in Figure 4d. RL, GL, GSP, YSP, BB, RKB, and PB did not show statistical differences or decrease ($p < 0.05$) among raw and heat-processed pulses. Moreover, for CC the extrusion enhanced the reducing power from an IC_{50} = 7.05 to 5.19 mg/mL, an increase of 26% after extrusion. Extruded and baked FB exhibited an increase of 18% and 29% in reducing power, respectively, in comparison with raw FB. For NB, only the baked sample showed a reducing power enhancement of 50%, from an IC_{50} = 7.19 mg/mL in raw NB to IC_{50} = 3.59 mg/mL in baked NB. In faba bean methanolic extracts from different varieties, Johnson et al. (2020) found that reducing power varied between samples finding a significant variance in the FRAP values when considered by

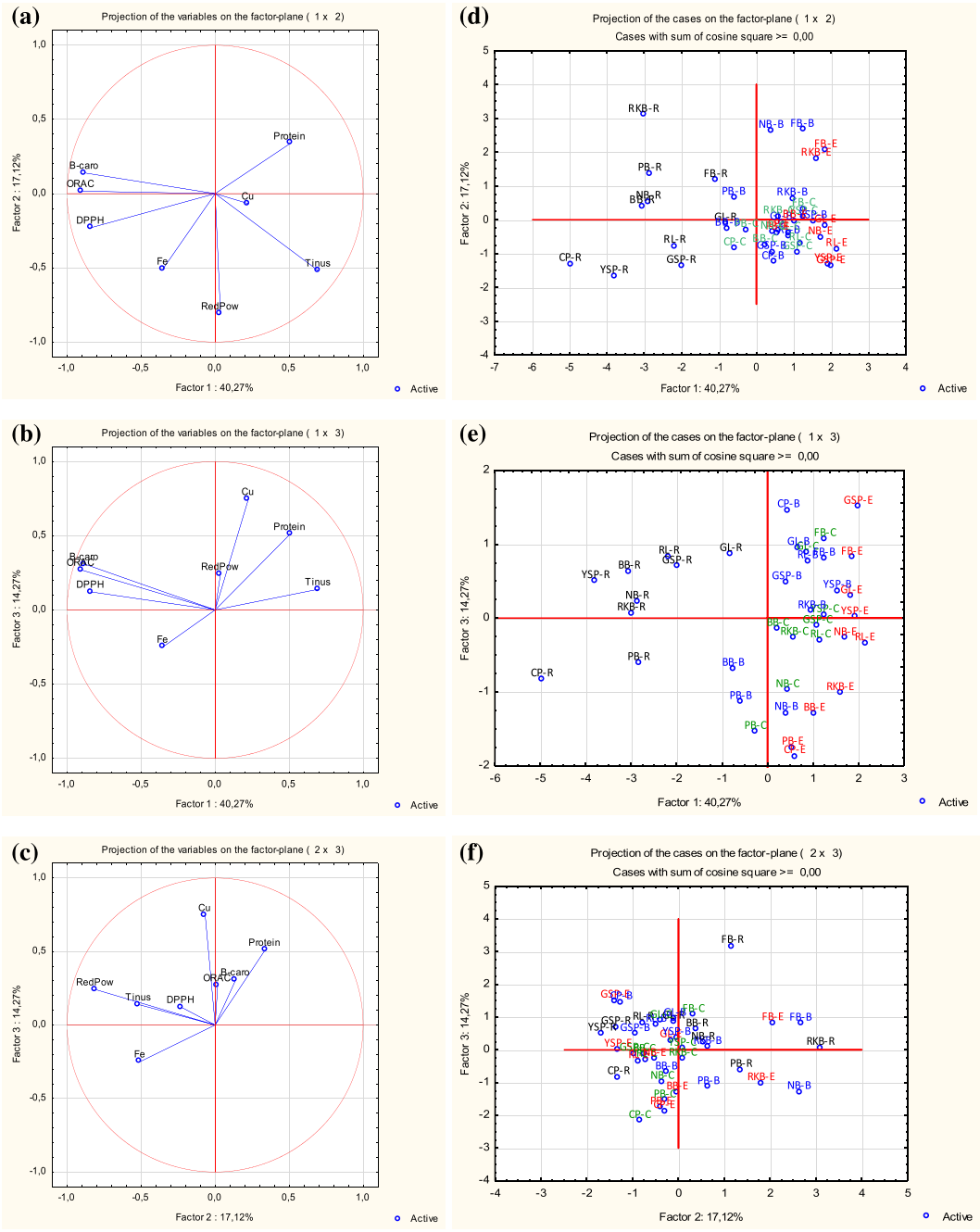

FIGURE 5 Principal component analysis (PCA) of protein content, protein digestibility, and antioxidant activities. (a–c) Positioning of variables on the first three factors of the PCA; (d–f) positioning of varieties by heat treatment on the first three factors of the PCA. Protein, protein content; Tinus, protein digestibility; DPPH (DPPH scavenging activity); Cu (Cu2 + chelating activity); Fe (Fe2 + chelating activity); RedPow (reducing power); B-caro (β-carotene bleaching activity); ORAC, oxygen radical antioxidant activity. RL (red lentil); GL (green lentil); GSP (green split pea); YSP (yellow split pea); CC (chickpea); FB (faba bean); RKB (red kidney bean); BB (black bean); PB (pinto bean); NB (navy bean). Suffixes -R, –E, -C and -B represent the heat treatments raw, extrusion, cooking and baking, respectively

variety. Amarowicz et al. (2009, 2010) found a higher reducing power in processed pulses than in crude samples. In most cases, they attributed this antioxidant capacity to phenolic compounds present in pulses, such as tannins (Dhull, Kaur, & Sandhu, 2020; Dhull, Punia, Kumar, et al., 2020).

3.5.4 | β-carotene bleaching activity

As with DPPH, β-carotene bleaching activity showed statistical improvements for the 10 processed pulses studied (Figure 4e). For all samples, extrusion showed the highest improvements of bleached β-carotene, with IC_{50} decreases of between 90% and 96% compared to raw pulses. On the other hand, the IC_{50} of cooked pulses showed an increased AOX from 30% to 75%, while baked pulses were enhanced by between 17% and 78% compared to raw samples. Processed pulse samples showed lower IC_{50} values of β-carotene bleaching activity than raw flours. In some pulses, β-carotene bleaching activity is related to the presence of phenolics, particularly (+)-catequin derivatives and other tannins (Amarowicz et al., 1996; Amarowicz et al., 2009; Amarowicz et al., 2010).

3.5.5 | ORAC

As observed for DPPH and β-carotene assays, ORAC test showed statistical differences ($p < 0.05$) among all raw and processed pulses. Interestingly, the highest ORAC IC_{50} decreases were found in extruded samples, ranging from 38% to 55%, followed by cooked RL, GL, GSP, and FB (24% to 42%) and baked YSP, CC, RKB, BB, PB, and NB (28% to 40%). Xu et al. (2007) reported ORAC values about 10 times higher in lentils than in green or yellow peas, as well as >20% lower than in beans. Processed FB, lentils, and peas did not show improvement in ORAC AOX compared to raw samples; the same effect was observed by Liu et al. (2020). The authors attributed this effect to phenolic releasing during any heat processing (boiling, pressure, microwave, and slow cooking). Moreover, in bioprocessed CC, Sánchez-Magaña et al. (2014) reported an enhancement of 64% in ORAC values, while in RKB and CC, ORAC improvements of 33% and 64% were found, respectively (Wu et al., 2012). Our results showed not so contrasting differences among raw pulses versus processed pulses.

4 | PCA

The PCA allowed us to detect similarities between samples and identify the main associations between variables responsible for the total variability of the data studied. The first two principal components (Figure 5), PC1 and PC2, represented 40.27% and 17.12%, respectively, of the total variance of the data. Through the analysis of PC1, it is possible to observe (Figure 5a): β-carotene, ORAC, and DPPH are three measurements that are similar and are especially related to factor 1 (strong negative correlation); reducing power is a measure rather independent of the previous ones and is mainly related to factor 2; Cu^{2+} is a measure rather independent of the previous ones and is mainly related to factor 3; Fe^{2+} is a measure rather independent of the previous ones and is mainly related to factor 4 (not illustrated); protein digestibility is mainly linked to factors 1 and 2; protein content is mainly related to factors 1, 3, and 5 (not shown); protein digestibility is inversely proportional to the IC_{50} of β-carotene, ORAC, and DPPH values (negatively correlated).

Figure 5 also shows the positioning of varieties and cooking methods on the first three factors of the PCAs. We noticed a clear separation between all the varieties without cooking (on the left on the Factor 1 to Factor 2 projection) (Figure 5d); in this group, we find the "beans" in the upper part of factor 2, the "lentils" around neutrality, and the "split peas" in the negative portion; the three heat processes form a fairly well-mixed whole (no clear trend); the F1–F3 (Figure 5e) projection confirms the grouping of grains without cooking on the negative side of the F1; this projection highlights the fact that "extruded" seems to be more to the right than the cooking and baking treatments. Therefore, the IC_{50} measured by β-carotene, ORAC, and DPPH (separation according to factor 1) are markedly higher for all the varieties in raw samples than for the cooked varieties. Between the uncooked varieties, we can differentiate the families ("beans" vs "split peas" vs "lentils") according to its IC_{50} measured by the reducing power method (factor 2). The F1–F3 projection indicates that the IC_{50} measured by β-carotene, ORAC, and DPPH (factor 1) are slightly lower for all varieties (more to the right according to factor 1).

5 | CONCLUSION

This work demonstrated that heat treatments have a positive effect overall on protein quality parameters. Processing can alter protein digestibility; IVPD of all samples was higher than 75%; additionally, extrusion and cooking improved EAA/TAA, BV, and PER in most pulses; however, baked beans showed the best IVPDCAAS values. Thus, the nutritional parameters of processed pulses indicated that these could be considered as good protein sources according to FAO/WHO (2013). Baking, cooking, and extrusion are recommended to reduce phytic acid and increase digestibility. Furthermore, the slight increase in protein digestibility could probably be explained by the low success obtained in eliminating and/or removing the bioactive compounds that are known to form complexes with proteins, leading to reduced accessibility for digestive enzymes action. The AOX was enhanced in most cases after heat treatments, especially after extrusion, which increased the β-carotene bleaching activity and ORAC values; reducing power was enhanced only in a couple of extruded and baked samples, while Cu^{2+} and Fe^{2+} presented a mixed behavior depending on pulse and varieties, unlike DPPH, where all treatments on the 10 pulses positively influenced the AOX. Saponins were more effectively reduced with cooking; phytic acid was decreased by extrusion and baking in beans, while for other pulses cooking and baking were more effective. Phenolics were decreased by cooking in most of cases. Finally, the IVPD was improved by all heating treatments, and IVPDCAAS was enhanced by baking in most of the pulses, but

extrusion was most effective for peas. Due to all mentioned above, choosing the best heat-processing treatment can raise the nutritional and antioxidant potentials in these selected pulses, which may be considered as excellent plant sources for developing functional and/or nutraceutical foods.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS STATEMENT

This manuscript does not contain any studies with human or animal subjects.

AUTHOR CONTRIBUTIONS

Oscar Abel Sánchez-Velázquez: Conceptualization, Methodology, Investigation, Data curation, and Writing-original draft. **Martin Mondor**: Validation and Editing. **Edith Oliva Cuevas-Rodríguez**: Supervision. **Sabine Ribéreau**: Writing-original draft, Methodology, and Investigation. **Yves Arcand**: Validation, Resources, and Data curation. **Alan Javier Hernández-Álvarez**: Conceptualization, Supervision, and Writing-review and editing.

DATA AVAILABILITY STATEMENT

Complete raw data and the processed data related to this publication are available with authors upon reasonable request.

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